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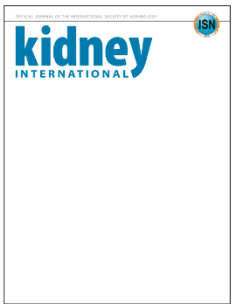

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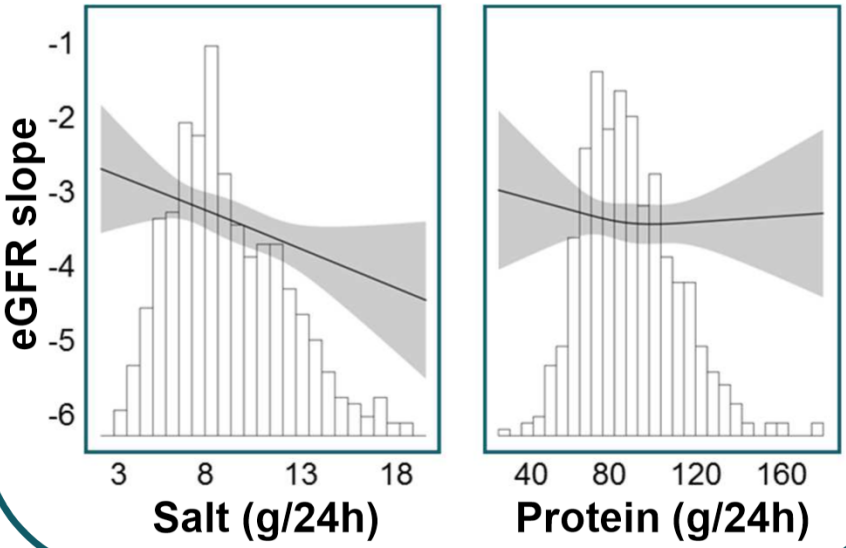
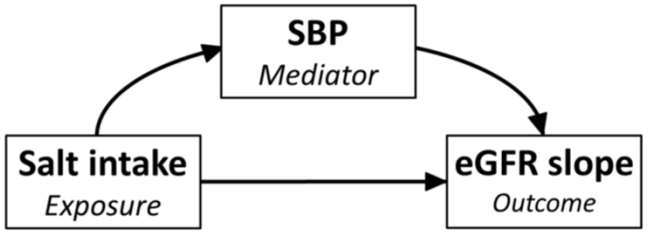
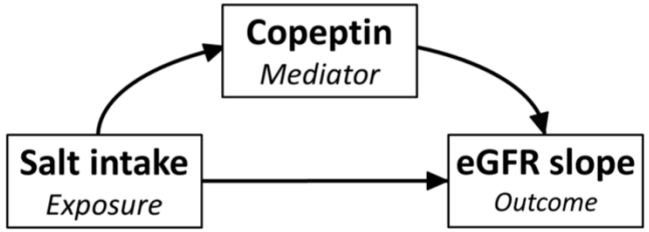
-0.55
Yearly eGFR change per 5 grams table salt
(p=0.02)

-0.00005
Yearly eGFR change per 5 grams protein
(p=0.9)





3% Mediation p=0.3



77% Mediation p<0.001

CONCLUSION:
Salt intake is associated with eGFR decline in ADPKD, the effect is mediated by plasma copeptin (surrogate for vasopressin).

Kramers et al, 2020

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[QUERY TO AUTHOR: title and abstract rewritten by Editorial Office – not subject to change]

Salt, but not protein intake, is associated with accelerated disease progression in autosomal dominant polycystic kidney disease.

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Abstract

In autosomal dominant polycystic kidney disease (ADPKD), there are only scarce data on the effect of salt and protein intake on disease progression. Here we studied association of these dietary factors with the rate of disease progression in ADPKD, and what the mediating factors are by analyzing an observational cohort of 589 patients with ADPKD. Salt and protein intake were estimated from 24-hour urine samples and the plasma copeptin concentration measured as a surrogate for vasopressin. The association of dietary intake with annual change in the estimated glomerular filtration rate (eGFR) and height adjusted total kidney volume (htTKV) growth was analyzed with mixed models. In case of significant associations, mediation analyses were performed to elucidate potential mechanisms. These patients (59% female) had a mean baseline age of 47, eGFR 64 mL/min/1.73m^2 and the median htTKV was 880 mL. The mean estimated salt intake was 9.1 g/day and protein intake 84 g/day. During a median follow-up of 4.0 years, eGFR was assessed a median of six times and 24-hour urine was collected a median of five times. Salt intake was significantly associated with annual change in eGFR of -0.11 (95% confidence interval (0.20 – - 0.02) mL/min/1.73m^2 per gram of salt, whereas protein intake was not (-0.00001 (-0.01 – 0.01) mL/min/1.73m^2 per gram of protein. The effect of salt intake on eGFR slope was significantly mediated by plasma copeptin (crude analysis: 77% mediation, and, adjusted analysis: 45% mediation), but not by systolic blood pressure. Thus, higher salt, but not higher protein intake may be detrimental in ADPKD. The substantial mediation by plasma copeptin suggests that this effect is primarily a consequence of a salt-induced rise in vasopressin.

Introduction

In chronic kidney disease (CKD), salt restriction is advocated to slow disease progression[1]. Salt restriction lowers blood pressure and potentiates renoprotective effects of RAAS-blockade[2]. The role of dietary protein restriction in slowing progression of CKD is more controversial, although several meta-analyses indicate a beneficial, albeit small effect[3, 4]. In ADPKD specifically, there are only scarce data on the renal effects of salt and protein intake.

In the CRISP cohort, an observational study in 241 ADPKD patients with early stage disease, higher urinary sodium excretion (indicating higher salt intake) was associated with more rapid kidney volume growth. In a post-hoc analysis of the HALT-PKD study, a randomized controlled trial in 1044 patients with later-stage ADPKD, sodium excretion was associated with steeper eGFR decline in patients with later-stage ADPKD but not in patients with early-stage ADPKD[5, 6]. It has been suggested that an association with eGFR decline may be caused by salt restriction potentiating the renal protective effects of RAAS-blockade, similar to non-ADPKD chronic kidney disease[6]. An alternative explanation could be that salt intake leads to accelerated disease progression in ADPKD by stimulation of vasopressin secretion. Vasopressin is known to be causally related to disease progression in ADPKD[7-9]. One of the main factors for vasopressin secretion is plasma sodium concentration[10], which increases after salt ingestion.

As urinary urea excretion was not measured in the HALT-PKD trial it is unclear whether protein intake was also associated with eGFR decline[6]. The effect of a low protein intake on rate of eGFR decline has been studied in a post-hoc analysis of the Modification of Diet in Renal Disease (MDRD) study, in which low protein diet was compared to a usual diet (study A) and a very low-protein diet was compared to a low protein diet (study B). In the subgroup of 200 ADPKD patients there were no significant differences in either sub-study, the results however were deemed

and protein intake and renal function decline in ADPKD. To address this aim, we analyzed data of ADPKD patients in a large observational cohort. We also aimed to study whether a potential association was mediated by vasopressin, or by other potential mechanisms.

Results

The cohort flow is detailed in **Figure 1**. The baseline characteristics are shown in **Table 1.** Mean age was 47±11 years, 59% was female, eGFR was 64±24mL/min/1.73m^2 and median htTKV was 880 mL (IQR 549 – 1352). There were no significant differences in age, sex, eGFR and htTKV in the 205 patients that were excluded due to insufficient follow-up data. Sodium excretion was 156±65 mmol/24h at baseline, corresponding with an estimated salt intake of 9.1±3.8 grams. Urea excretion was 390±132 mmol/24h, corresponding with an estimated protein intake of 84±25 grams. Sodium excretions and urea excretion during all visits in the DIPAK 1 trial and the DIPAK observational cohort are shown **Figure 2**.

During a median follow-up time of 4.0 years (IQR 2.6 – 5.0) eGFR was assessed 6 times (IQR 5–14) and 24-hour urine was collected 5 times (IQR 4–7). Average annual change in eGFR was -3.50 mL/min/1.73m^2 per year (95% CI -3.70 – -3.29).

Sodium excretion and urea excretion

Sodium excretion was strongly correlated with urea excretion (St. β = 0.61, unstandardized B = 1.8 mmol urea per mmol sodium (95% CI 1.6 – 2.0), p<0.001). In mixed model analysis, sodium excretion was univariably associated with change in eGFR (-0.16 mL/min/1.73 m^2 per year per 18 mmol of sodium, (95%CI -0.24 – -0.08) p<0.001), as was urea excretion(-0.03 mL/min/1.73 m^2 per year per 40 mmol of urea, (95%CI -0.05 – -0.001) p=0.04). In multivariable analysis, adjusted for age, sex, body surface area (BSA), baseline htTKV and DNA mutation the association of sodium excretion with change in eGFR remained statistically significant (**Table 2**). In contrast, the association between urea excretion and eGFR slope lost significance after adjustment for potential confounders (**Table 2**).

Figure 3 graphs the relationship between sodium excretion and urea excretion and eGFR slope. Based on the excretions of sodium and urea we estimated salt and protein intake. In the

eGFR was not significant (-0.00001 mL/min/1.73 m2 per year per gram protein, (95%CI -0.01 – 0.01, p=0.9) (**Supplementary Table S1A**). When we excluded the patients that used Lanreotide during the DIPAK 1 trial the results were essentially the same (**Supplementary Table S1B**).

In univariate analysis, both sodium excretion and urea excretion were associated with htTKV growth (0.63% per year per 18 mmol sodium (95%CI 0.40 – 0.87, p<0.001) and 0.18% per year per 40 mmol urea (95%CI 0.09 – 0.28, p<0.001), respectivel). The association of sodium excretion with htTKV growth remained significant after adjustment for age, sex, BSA, baseline htTKV and DNA mutation whereas the association of urea excretion lost significance (**Table 3**).

Sensitivity analyses

As sensitivity analyses we repeated the linear mixed model analyses with salt and protein intake per kg ideal bodyweight, and salt and protein per kg actual bodyweight (**Supplementary Tables 1C and D**). As additional sensitivity analyses we repeated the analyses using baseline 24-hour excretions instead of mean excretions, we subsequently used median excretions instead of mean excretions. We excluded 24-hour urine collections if the 24-hour creatinine excretion was >30% different from that participant's average creatinine excretion. Finally, we performed a sensitivity analysis in which we adjusted for albuminuria. All of these analyses yielded essentially the same results.

Sub-group analysis

We tested for differences in the association between salt intake and annual change in eGFR across several subgroups **(Figure 4).** Higher salt intake was associated with more rapid eGFR decline, or neutral in all subgroups. The interaction term between use of RAAS-blockade and salt intake was significant (p=0.02), with a stronger negative association in patients who did not use RAAS-blockade. There was a trend towards a significant interaction with age (p=0.06) and baseline eGFR (p=0.07). Compared to patients that did not use RAAS-blockade, RAAS-blockade users had similar salt intake,

compared to older patients (9.0 ± 2.7 grams vs. 8.3 ± 2.5 grams, p=0.002). Salt intake was similar in patients with higher eGFR (8.8 ± 2.8 grams) and patients with lower eGFR (8.4 ± 2.5 grams), p=0.07 .

Mediation by blood pressure, RAAS or copeptin

We performed structural equation models to test for possible mediators of the association of excretion and eGFR slope. First, we tested whether the effect was mediated by an effect on blood pressure. In this model, the total effect of estimated salt intake on eGFR slope was estimated as -0.13 mL/min/1.73 m2 per year per gram of table salt, (95%CI -0.23 – -0.02) p=0.03. The direct effect of blood pressure on eGFR slope was significant (-0.02 mL/min/1.73 m2 per year per mmHg, (95%CI -0.03 – -0.01) p=0.02). However, the direct effect of salt intake on systolic blood pressure was insignificant (p=0.3). Thus, the indirect effect through systolic blood pressure was not significant (estimate = -0.005 (95%CI -0.01 – 0.003), p=0.3). Ergo, there was no significant mediation by systolic blood pressure.

We tested whether the effect of salt intake on eGFR was mediated by plasma plasma renin or plasma aldosterone in patients that did not use RAAS blockade (n=58). In these patients median plasma renin was 10.6 pg/mL (IQR 6.5 – 16.7), median plasma aldosterone was 265 pg/mL (IQR 181 – 363). Both indirect effects were not significant (p=0.3 and p=0.4 respectively), indicating there was no statistically significant mediation by plasma renin or plasma aldosterone.

Next, we investigated whether the association of sodium excretion and eGFR slope was mediated by copeptin (average of two values) . There was high correlation between the two plasma copeptin measurements (spearman coefficient 0.85, p<0.001, **Supplementary figure 1**). In a crude model the total effect of salt intake on eGFR slope was estimated as -0.16 (95%CI -0.23 - -0.09) mL/min/1.73 m2 per year per gram of table salt, p<0.001. The indirect effect, mediated by copeptin, was estimated to be -0.12 (95%CI -0.18 - -0.08) mL/min/1.73 m2 per year per gram of table salt,

After this crude analysis, the mediation model with copeptin was adjusted for potential confounders. In multivariable analysis baseline age, sex and eGFR were significantly associated with plasma copeptin on top of estimated salt intake. Sex and DNA mutation were significantly associated with eGFR slope on top of either salt intake or plasma copeptin. After adjustment for these variables, the total effect of salt intake on eGFR slope was -0.14 (-0.23 - -0.04) mL/min/1.73 m^2 per year per gram of table salt (p=0.004), indirect effect was -0.06 (-0.10 – -0.02)), p=0.004 (**Figure 5**). Thus, in the adjusted analysis, the effect of salt intake on eGFR slope is mediated by copeptin by 45% (95%CI 1 – 89%). There was no indication of an impact of unmeasured confounding on these results (**Supplementary table 3**).

We repeated the mediation analysis with htTKV slope as dependent variable. The total effect of salt intake on htTKV slope was 0.59% htTKV growth per year per gram of table salt, (95%CI 0.33 – 0.86) p=<0.001. There was no significant mediation by systolic blood pressure (p=0.5). The indirect effect, mediated by plasma copeptin, was statistically significant: 0.15% htTKV growth per year per gram of table salt, (95%CI 0.04 – 0.25) p=0.006. Thus the effect of salt intake on htTKV slope is mediated by plasma copeptin by 25% (95%CI 4 – 45).

We performed an exploratory mediation analysis to evaluate whether the effect of copeptin on eGFR slope was in turn mediated by htTKV growth. Rate of htTKV change was significantly associated with rate of eGFR change -0.38% per mL/min/1.73m^2, p<0.001. In the crude analysis the total effect of LN transformed plasma copeptin on eGFR slope was -1.51 (95%CI -1.88 – -1.14, p<0.001), the indirect effect through htTKV growth was -0.20 (95%CI -0.34 – -0.05, p=0.008), ergo 13% (95% CI 3% – 23%) mediation. However, after adjustment for confounders this mediation lost statistical significance with an indirect effect of -0.12 (95%CI -0.26 – 0.02, p=0.09), 8% mediation (95%CI -2% – 8%).

Discussion

In this study, we found that salt, and not protein intake is associated with kidney function decline in ADPKD. The effect is significantly mediated by plasma copeptin level, suggesting that salt intake may have detrimental effects by increasing vasopressin.

The association of salt intake with eGFR decline is in line with scarce earlier findings. An association between sodium excretion and ADPKD disease progression was first shown in the CRISP cohort, where a significant association with kidney volume growth was found[13]. In multivariable analysis a significant association with eGFR decline was not found. However, this was a cohort of patients with early-stage ADPKD where GFR had not yet started to decline. Finding associations with rate of eGFR decline in such a cohort is difficult. Sodium excretion in the CRISP cohort was higher than in our cohort (193±86 vs 156±65 mmol/24h). Similarly, in a post-hoc analysis of the HALT-PKD study A, which included patients with early-stage ADPKD, the association between salt intake and eGFR decline was not significant (p=0.09). Conversely, in HALT-PKD study B (that included patients with later-stage ADPKD, average sodium excretion 178±80) the association of salt intake with eGFR decline did point towards beneficial effects of salt restriction.[6] We were able to confirm this finding in a cohort of ADPKD patients with a wide range of renal function. Within the present cohort, there was significantly higher average salt intake (and variance) in the younger patients compared to older patients. This may have contributed to finding a trend towards a significantly stronger association between salt intake and eGFR decline within this sub-group.

In theory, protein intake could be detrimental through vasodilatory effects that cause intraglomerular hyperfiltration or through increment in vasopressin [14-16]. To our knowledge, only two studies assessed the association between protein intake and eGFR decline in ADPKD. Similar to sodium excretion, in the CRISP cohort no significant association between urea excretion and kidney function decline could be shown[5]. A post-hoc analysis of the MDRD study also did not show a

caused acute lowering of GFR. This effect may have negated a subsequent beneficial effect of slower GFR decline because the follow-up was not long enough[11]. Furthermore, the authors suggest that they may have lacked power to show the effect[11]. One advantage of the observational nature of our study is the lack of an acute effect of a diet intervention. Therefore, an acute hemodynamic effect cannot be the explanation why we did not find an association with kidney function decline. While we cannot strictly exclude lack of power as an explanation for not finding an association between protein intake and eGFR decline, there also did not seem to be a trend towards a positive association after adjustment for potential confounders, which makes it less likely.

A possible mediating mechanism of the effect of salt intake on disease progression could be through blood pressure. Salt-sensitive hypertension is common in chronic kidney disease. Furthermore, the HALT-PKD trial has shown beneficial effects of rigorous blood-pressure control regarding TKV growth in ADPKD, and also regarding eGFR decline in the sub-group of patients with most severe disease[17, 18]. Indeed, in in our study there was a negative association between blood pressure and eGFR decline. However, we were not able to show a significant association between salt intake and blood pressure. In line, the mediation analysis did not show significant mediation by systolic blood pressure.

Another mechanism that has been suggested to underlie a beneficial effect of restricted salt intake on ADPKD disease progression is potentiation of RAAS-blockade[6]. A post-hoc analysis of the IDNT and RENAAL trials showed that lower dietary sodium intake enhances the beneficial effects of RAAS-blockade in 1137 patients with diabetic nephopathy[19]. The notion that salt restriction potentiates the renal protective effects of these medicaments is widely accepted, nowadays. However, if potentiation of RAAS-blockade would be the mechanism of effect in ADPKD, we would expect a stronger beneficial effect of salt restriction in RAAS-blockade users than in patients who did not use RAAS-blockade. As shown in figure 4 this was not the case; the association with salt intake

restriction in ADPKD. We were not able to show significant mediation effect of either renin or aldosterone in patients that did not use RAAS blockade, however, we lacked sufficient power to draw definite conclusions.

We did find significant mediation of the salt intake effect by copeptin, the surrogate marker of vasopressin. It is known that an increase in salt intake will cause an increase in plasma osmolality, triggering vasopressin secretion[20]. Vice versa, a short-term pilot study in 34 ADPKD patients by *Amro et al.* showed that a combined salt and protein restriction in combination with adjusted water intake led to reduction in vasopressin secretion[21]. In CKD in general, vasopressin can cause relative glomerular hyperfiltration that is potentially detrimental[22]. In ADPKD specifically, vasopressin causes cystogenesis and is associated with GFR decline[21]. Treatment with antagonists of the vasopressin V2 receptor ameliorate kidney volume growth and eGFR decline[8, 9]. In the present study, not finding significant mediation of the plasma copeptin effect on eGFR decline by htTKV growth suggests that the detrimental effect of copeptin may not primarily have been a consequence of cyst growth. Mediation of the salt effect on eGFR decline by vasopressin could also explain not finding an independent effect of protein intake. Experiments have shown that urea is an ineffective osmole in plasma, i.e. that infusion of sodium causes a much greater increase in vasopressin secretion than an equal infusion of osmoles of urea[20]. If urea does not have an effect on vasopressin, and the detrimental effects of dietary factors in ADPKD are mainly through vasopressin, one would not expect a detrimental effect of protein intake.

There are limitations to this study that need to be addressed. Due to the lack of a standardized diet there probably were variations in salt and protein intake within subjects during the study, which may obscure associations with rate of disease progression. For that reason we used the average values of all 24-hour urines that were collected throughout the study. The average excretions are probably a reasonable measure of average intake. Furthermore, due to the

The main strengths of this study include frequent follow-up visits, allowing for accurate eGFR slope estimations, and estimation of protein and salt intake by gold standard measures, i.e. by collection of multiple 24-hour urine samples over the entire study period. Previous studies have shown that multiple 24-hour collections are necessary to obtain accurate associations, we obtained a median of 5 (IQR 4–7) samples per patient[23]. Finally, this is the first study that shows an association between salt intake and eGFR slope in a cohort where early-stage and later-stage ADPKD patients are both well represented.

Our finding that one gram of salt intake is associated with -0.11 mL/min/1.73m^2 annual change in eGFR suggests that adherence to the current sodium restriction guidelines could significantly postpone end-stage kidney disease (ESKD). If a patient of 30 years old, with an eGFR of 110 mL/min/1.73m^2 would adhere to the currently advised maximum of 5 grams of table salt per day, instead of 9.1 grams (average in this cohort), he would hypothetically ameliorate his annual change in eGFR from -3.50 mL/min/1.73m^2 per year (average in this cohort) to -3.05 mL/min/1.73m^2 per year. This hypothetical patient would postpone ESKD by 4 years, from age 57 to 61. Of course this hypothesis needs confirmation in an intervention study. As this cohort included few patients with very low estimated salt intake, we cannot investigate the potential effect of lower sodium intake and therefore cannot make a recommendation for lower sodium restrictions than the current guidelines. Based on our data there is no indication that protein restriction is beneficial. Additional benefits of salt restriction could be reduction of polyuria both in late-stage ADPKD and in patients using the vasopressin V2 receptor antagonist tolvaptan. In both cases osmolar excretion is the main determinant of urine volume due to urine concentrating defects[24]. Whether protein intake and salt intake have the same effects on reduction of polyuria remains the subject of future studies.

In conclusion, this study shows that 24-hour urinary sodium excretion is associated with the rate of eGFR decline in ADPKD and suggests that salt restriction should be an important focus of

Methods

For this study, we used the data of the DIPAK observational cohort study that was designed to investigate the natural course of polycystic kidney disease. This cohort study was initiated to continue follow-up of ADPKD patients that participated in the DIPAK-1 randomized controlled trial in which the renoprotective effect of the somatostatin analogue lanreotide was assessed (n=305). Inclusion into the observational cohort was extended to ADPKD patients from the outpatient clinic (n = 489) and is still ongoing. Data were collected in the University Medical Centers of Groningen, Leiden, Nijmegen and Rotterdam. Design, methods and the main outcomes of the DIPAK 1 trial have been published elsewhere[25, 26]. In brief, patients were included between 2012 and 2015 if they were 18-60 years of age, had ADPKD (modified Ravine criteria[27]), and an eGFR between 30-60 mL/min/1.73m^2. After a baseline visit, patients were seen after 4, 8, 12, 48, 96, 120 and 132 weeks, and blood was collected every 12 weeks. After the end of the trial, 175 patients agreed to continue follow-up. Inclusion criteria for the observational cohort study were age of ≥18 years and an eGFR ≥15 mL/min/1.73m^2. All eligible patients that were seen at the outpatient clinic until December 2017 of the four centers were asked to participate in the observational study. Contraindications for participation in the trial and the observational cohort were concomitant diseases or medication use that may influence the natural course of ADPKD (e.g. diabetes mellitus or chronic NSAID use). For the present analyses we included ADPKD patients with a minimal number of estimated glomerular filtration rate (eGFR) assessments of three during at least two years of follow-up, leaving n=589 patients for analysis (STROBE flow diagram, **Figure 1**). The DIPAK observational study was approved by the Institutional Review Board of the University Medical Center Groningen and conducted in adherence to the ICH-GCP (International Conference on Harmonization-Good Clinical Practice) guidelines. Written informed consent was obtained from all patients.

eGFR was estimated using the creatinine based CKD-EPI formula [28]. Fasting plasma copeptin concentrations were measured using a sandwich immunoassay (Thermo Fisher Scientific BRAHMS, Hennigsdorf/Berlin, Germany), at baseline in all patients and also at week 12 in the DIPAK-1 patients. Renin (Renin III Generation RIA Cisbio Bioassays, Codelet, France) and aldosterone (Demeditec Diagnostics GmbH, Kiel, Germany) were measured at baseline by radioimmunoassay. Osmolality was measured by the freezing point depression method, sodium and potassium concentration by ion specific electrodes, and urea by an enzyme kinetic assay. Magnetic Resonance Imaging (MRI) was performed using a standardized MRI protocol without the use of intravenous contrast. Total kidney volume (TKV) was assessed by manual tracing of T2-weighted coronal magnetic resonance images using Analyze direct 9.0 software (AnalyzeDirect, Inc., Overland Park, KS).

24-hour urine

24-hour urine samples were collected at baseline, at week 12, 48, 96, 120, 132 and in case of early end of treatment during the DIPAK 1 trial, and yearly thereafter. For all analyses the average values of all available 24-hour urine samples were used. Sodium was measured by ion specific electrodes, urea by enzyme kinetic essay. Salt intake was estimated by multiplying sodium excretion by sum of the molar mass of sodium and chloride: 'Salt intake = sodium excretion (mol) x (22.99 + 35.45)'. Total protein intake was estimated from urea excretion by the method of Maroni et al[29]: 'Protein intake = (Urea excretion (mmol) * 0.028 + 0.031*body weight (kg)) *6.25'.

Statistical analyses

For statistical analyses we used SPSS version 23 (SPSS Inc), or Stata SE 14 in case of linear mixed-model analyses. For all analyses a two-sided $P < 0.05$ was considered statistically significant.

Mixed-model repeated measure analyses were used to evaluate associations of dietary

Intercept and slope were allowed to vary randomly, with an unstructured covariance matrix. Fixed effects in the models were time, sodium excretion (or estimated salt intake), body surface area (BSA), age, sex, htTKV and DNA mutation and the interactions of these variables with time. A significant interaction time*sodium excretion signifies an association with annual eGFR decline. Similar analyses were performed for urea excretion (or estimated protein intake). Patients were included in the analysis if all data was available list-wise (complete case analysis). Follow-up MRIs were performed during the DIPAK 1 trial. Change in height adjusted TKV (htTKV) was assessed using log_{10}-transformed htTKV data, the antilog of the estimated effect was derived from the mixed-model analysis to provide annual percentage change of htTKV. Model validation was performed by visual inspection of the residual plots. We plotted histograms of the level 1 residuals and histograms of residuals of random slopes and intercepts. Standardized residuals were plotted versus predicted values and time.

We performed a number of sensitivity analyses. Salt and protein intake were corrected for actual bodyweight and ideal body weight. Ideal body weight was derived using a BMI of 22 kg/m^2 as reference. Furthermore, the analyses were repeated excluding the 142 patients who received Lanreotide treatment during the DIPAK 1 trial. We also performed sensitivity analyses to investigate the effect of urine collection errors. We repeated the analyses with median urinary excretion values instead of the mean, and we excluded follow-up urine collections if creatinine excretion was >30% different from the mean. Finally, we performed a sensitivity analysis in which we used baseline 24-hour urine collections instead of average, and we performed a sensitivity analysis in which we adjusted for albuminuria.

Structural equation models (SEM) were used to perform mediation analysis with eGFR slope as an outcome variable, estimated salt intake as an exposure and systolic blood pressure,plasma copeptin, plasma renin and plasma aldosterone as potential mediators. For copeptin, the average of

use RAAS-blockade. eGFR slope and intercept were added as latent variables. In contrast to longitudinal mixed effects models, latent growth structural equation models require time-structured data (i.e. data collected at the same time from baseline for every subject). Therefore, data from the DIPAK observational study could not be combined with data from the DIPAK 1 trial, and only the data collected during the DIPAK 1 trial was included for this analysis. The same analysis was repeated with htTKV growth as outcome variable.

In case of significant mediation effect on eGFR slope, we investigated the role of potential measured and unmeasured confounding. As potential confounders we evaluated age, sex, body surface area, baseline eGFR, baseline TKV (log10 transformed), DNA mutation (PKD1 or PKD2), use of RAAS-blockade and use of diuretics. We evaluated exposure-mediator confounding, mediator-outcome confounding and exposure-mediator confounding. Using structural equation models we estimated univariable associations of potential confounders with mediator and outcome. Subsequently, we added all potential confounders that were univariably associated to a multivariable model. Variables that were associated with exposure or outcome in multivariable analysis ($p<0.05$) after backwards elimination were included in the SEM mediation model. The impact of unmeasured confounding was evaluated by a series of sensitivity analyses, by method of Imai, Keele and Yamamoto[30].

Sub-group analyses were performed for the association between salt intake and eGFR slope by including an interaction term between subgroup and salt intake (Salt intake x Subgroup) to the multivariable mixed model, adjusted for age, sex and BSA. If the interaction term was significant, the subgroup was considered a significant moderator for the association.

Disclosures

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Supplementary material

Supplementary Table 1. Sensitivity analyses: associations with slope of estimated GFR

Supplementary table 2. Baseline characteristics of patients that use RAAS blockade and patients that do not use RAAS-blockade.

Supplementary table 3. Sensitivity analysis mediation.

Supplementary figure 1. Scatterplot of copeptin measurement at baseline and at week 12.

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Table 1. Baseline characteristics

	n=589
Age (years)	47±11
Sex (female)	245 (59%)
Weight females (kg)	76±15
Weight males (kg)	90±14
Height females (m)	1.70±0.07
Height males (m)	1.84±0.07
SBP (mmHg)	133±14
DBP (mmHg)	82±10
RAAS-blockade (yes)	415 (71%)
eGFR (mL/min/1.73m^2)[a]	64±24
htTKV (mL/m)	880 (549-1352)
Copeptin (pmol/L)	7.6 (4.5 – 13.2)
MAYO risk class	
1A/1B (low risk disease)	145 (26%)
1C/1D/1E (high risk disease)	385 (69%)
2 (atypical)	27 (5%)
PKD genotype	
PKD1 truncating	241 (42%)
PKD1 non-truncating	151 (26%)
PKD2	128 (22%)
Unknown/Not detected	50 (9%)
Sodium excretion (mmol/24h)	156±65
Estimated salt intake (g/24h)	9.1 ± 3.8
Urea excretion (mmol/24h)	390 ± 132
Estimated protein intake (g/24h)	84 ± 25
Urine volume (L/24h)	2.3 ±0.8

Variables are presented as mean ± SD, as median [interquartile range] in case of non-normal distribution or as percentage for categorical variables.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; htTKV, height-adjusted total kidney volume.

[a]Estimated by CKD-EPI equation.

ciations of sodium and urea excretion with eGFR slope (n=553).

nd urea excretion vs. eGFR slope (mL/min/1.73m^2 per year)						
	Model 1		Model 2		Model 3	
	Est. (95% CI)	P	Est. (95% CI)	P	Est. (95% CI)	P
xcretion (per 18 mmol)*	**-0.12 (-0.20 – -0.03)**	**0.006**			**-0.11 (-0.21 – -0.02)**	**0.02**
etion (per 40 mmol)*			-0.02 (-0.05 – 0.01)	0.2	-0.002 (-0.03 – 0.03)	0.9
ear)*	0.01 (-0.01 – 0.03)	0.4	0.01 (-0.01 – 0.03)	0.3	0.01 (-0.01 – 0.03)	0.4
le)*	-0.08 (-0.56 – 0.40)	0.7	-0.01 (-0.50 – 0.47)	0.9	-0.08 (-0.57 – 0.40)	0.7
m^2)*	0.07 (-1.05 – 1.20)	0.9	-0.16 (-1.28 – 0.97)	0.8	0.08 (-1.06 – 1.22)	0.9
KV (mL/m)	**-2.98 (-3.70 – -2.27)**	**<0.001**	**-3.05 (-3.76 – -2.33)**	**<0.001**	**-2.99 (-3.70 – -2.27)**	**<0.001**
ation (Ref: PKD2)						
uncating	**-1.25 (-1.78 – -0.72)**	**<0.001**	**-1.24 (-1.77 – -0.71)**	**<0.001**	**-1.25 (-1.78 – -0.73)**	**<0.001**
on-truncating	**-1.18 (-1.73 – -0.62)**	**<0.001**	**-1.14 (-1.70 – -0.58)**	**<0.001**	**-1.18 (-1.73 – -0.62)**	**<0.001**
n	-0.64 (-1.33 – 0.05)	0.07	-0.64 (-1.33 – 0.06)	0.07	-0.64 (-1.33 – 0.06)	0.07

s and p-values shown for the interactions of variables with time. The interaction with time signifies the effect of said variable on eGFR over time, i.e. the effect on eGFR
el 1 shows the association of sodium excretion with eGFR slope. Model 2 shows the association of urea excretion with eGFR slope. Model 3 shows the associations of
urea excretion with eGFR slope in the same model. All models were adjusted for time, age, sex, BSA, and their interactions with time. The estimations for the variables
ed with time (not shown) signify the effect of said variable on baseline eGFR (the intercept).
ns: BSA, body surface area.

ciation of estimated salt intake and protein intake with annual htTKV growth (n=283)

rea excretion vs. htTKV growth (% per year)						
	Model 1		Model 2		Model 3	
	Est. (95% CI)	P	Est. (95% CI)	P	Est. (95% CI)	P
tion (per 18 mmol)*	**0.31 (0.09 – 0.53)**	**0.007**			**0.44 (0.18 – 0.71)**	**0.001**
n (per 40 mmol)*			0.01 (-0.07 – 0.09)	0.8	-0.09 (-0.19 – 0.01)	0.09
)*	-0.05 (-0.12 – 0.02)	0.2	-0.07 (-0.14 – 0.00)	0.05	-0.05 (-0.12 – 0.02)	0.2
*	**-2.87 (-3.94 – -1.79)**	**<0.001**	-3.09 (-4.13 – -2.04)	<0.001	-3.01 (-4.05 – -1.95)	<0.001
*	0.37 (-2.36 – 3.18)	0.9	1.55 (-1.11 – 4.29)	0.3	0.75 (-1.93 – 3.51)	0.6
e htTKV (mL/m)	**0.46 (-1.32 - 2.27)**	**0.6**	**0.58 (-1.14 – 2.32)**	**0.5**	**0.40 (-1.32 – 2.14)**	**0.7**
n (Ref: PKD2)						
ating	**-1.38 (-2.64 – -0.10)**	**0.03**	**-1.48 (-2.70 – -0.25)**	**0.02**	**-1.41 (-2.63 – -0.17)**	**0.03**
runcating	-0.92 (-2.29 – 0.48)	0.2	**-1.08 (-2.40 – 0.26)**	**0.1**	**-0.85 (-2.19 – 0.50)**	**0.2**
	-0.77 (-2.56 – 1.05)	0.4	-0.90 (-2.63 – 0.86)	0.3	-0.77 (-2.50 – 1.00)	0.4
on group (Lanreotide)	**-2.07 (-2.93 – -1.20)**	**0.004**	-2.15 (-3.00 – -1.30)	<0.001	-2.21 (-3.05 – 1.35)	<0.001

d p-values shown for the interactions of variables with time. The interaction with time signifies the effect of said variable on eGFR over time, i.e. the effect on eGFR hows the association of sodium excretion with eGFR slope. Model 2 shows the association of urea excretion with eGFR slope. Model 3 shows the associations of excretion with eGFR slope in the same model. All models were adjusted for time, age, sex, BSA, and their interactions with time. The estimations for the variables th time (not shown) signify the effect of said variable on baseline eGFR (the intercept).
SA, body surface area.

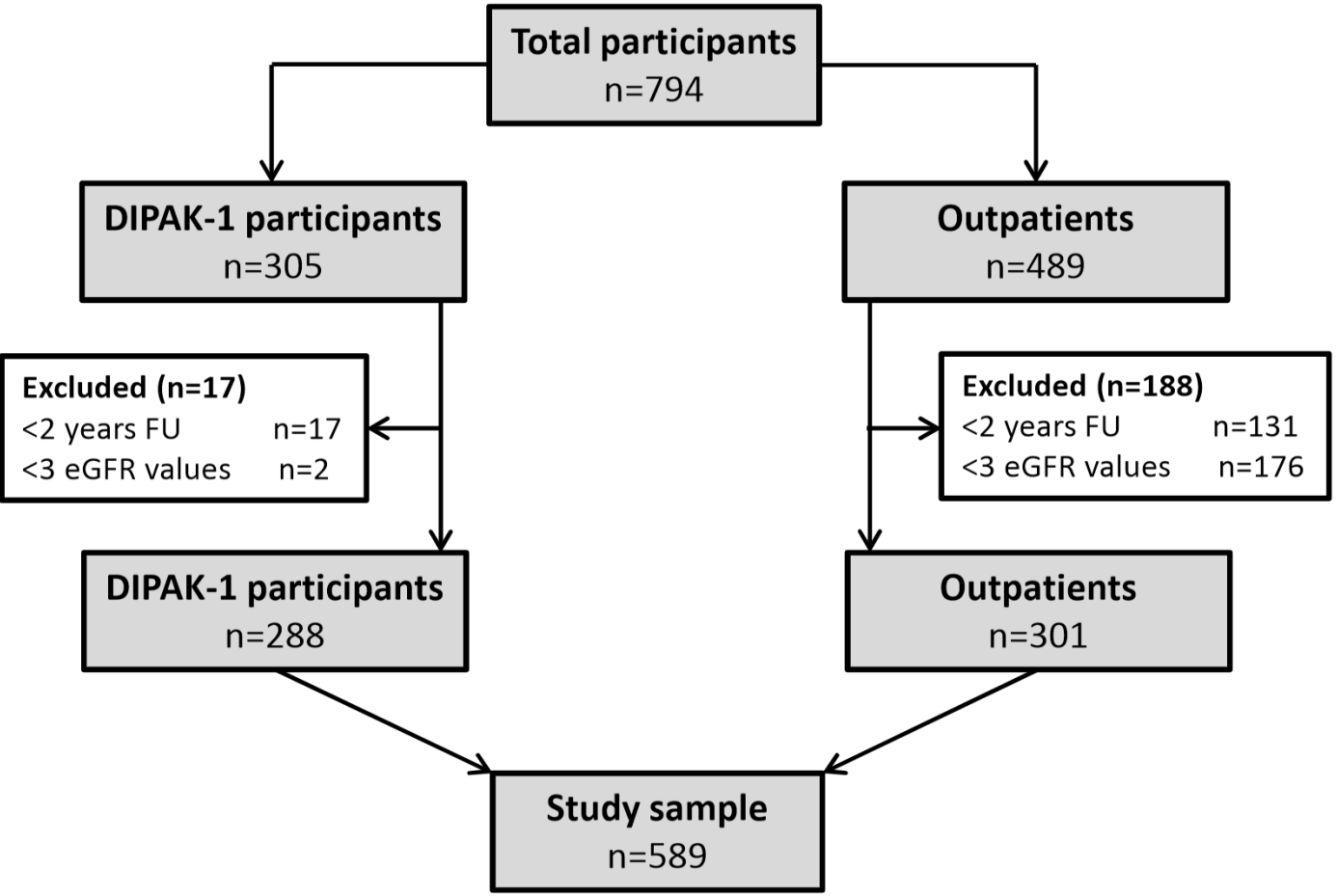
DBE flow diagram

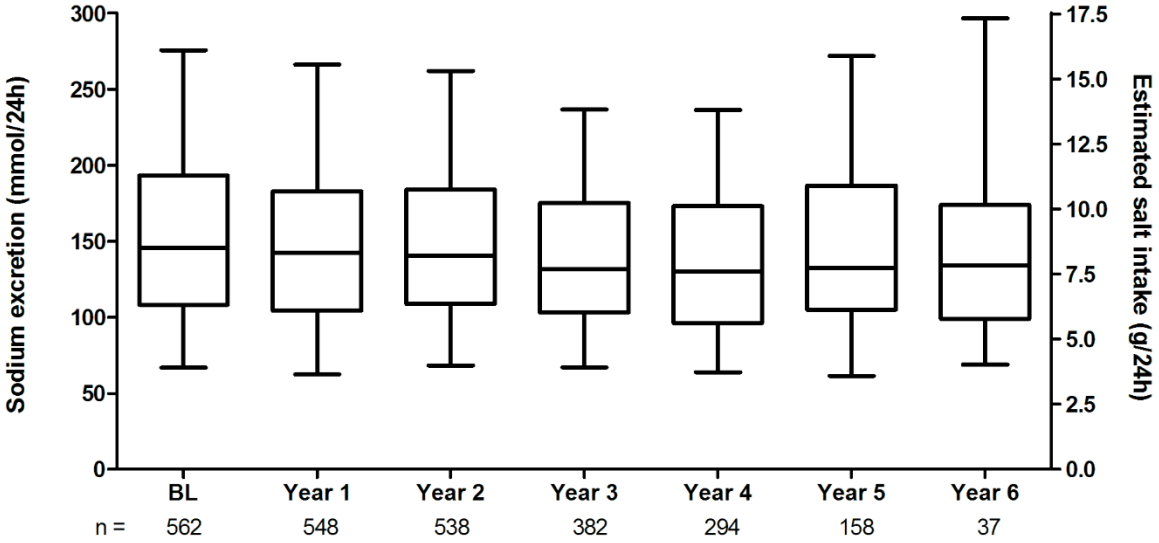
n sodium excretion and urea excretion at the yearly visits, with estimated salt and protein intake.
cate 5% -95% range

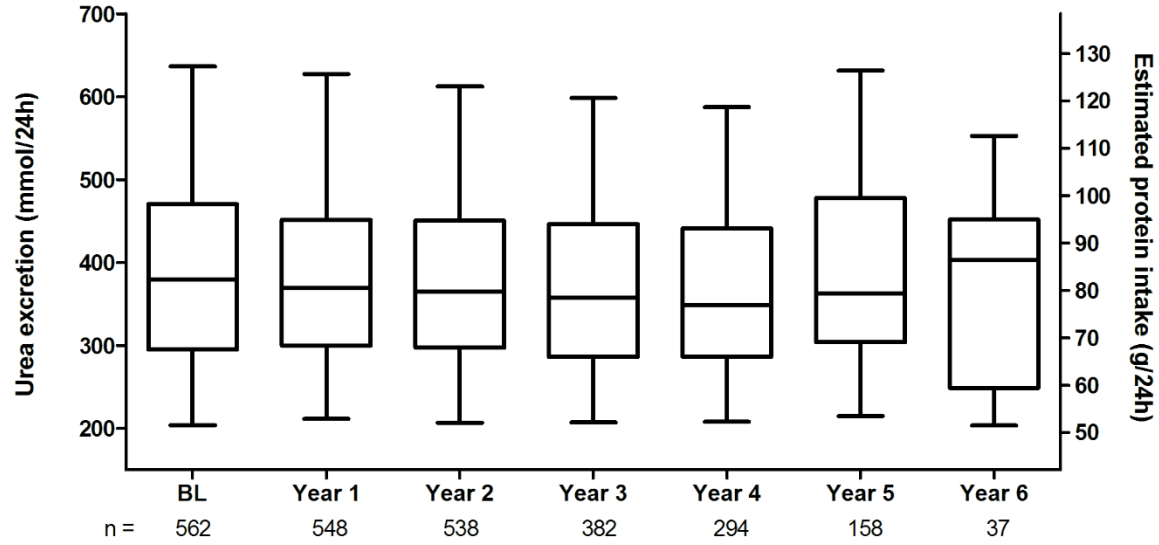
ribution of sodium excretion and urea excretion and their association with eGFR slope

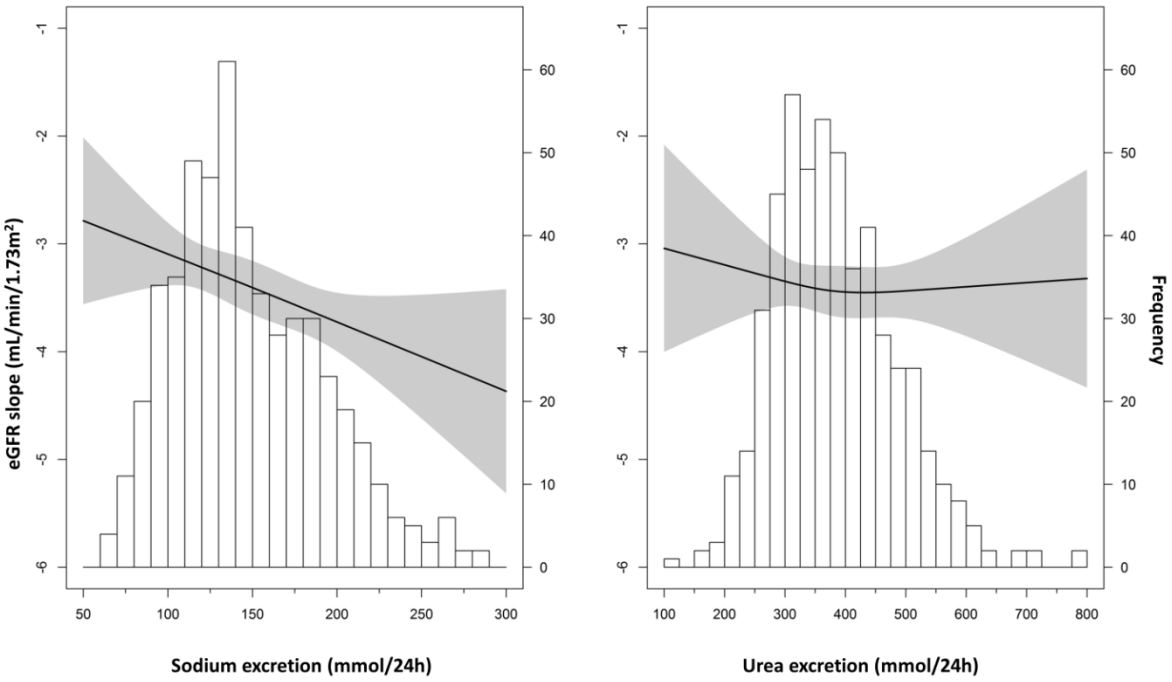
ciation of salt intake with slope of estimated GFR (mL/min/1.73 m^2 per year) in subgroups.

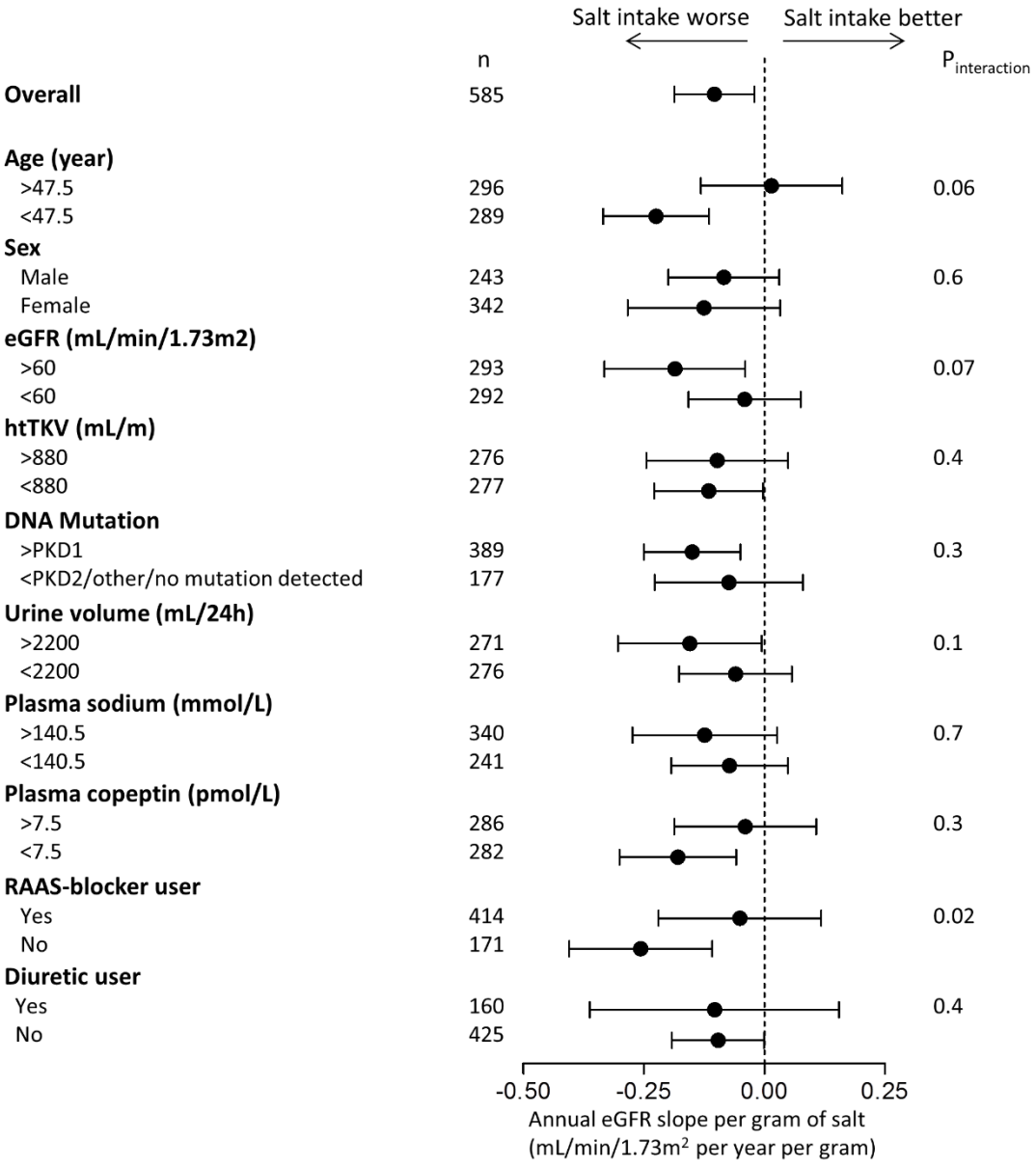
liation analyses of the effect of sodium excretion on eGFR slope
es are Ln-transformed. The total effect of salt intake on eGFR slope is estimated as -0.14 mL/min/1.73m^2 per year per gram of table salt. The
of salt intake (per gram) on eGFR slope (mL/min/1.73m^2 per year) via copeptin (pmol/L) is -0.06 mL/min/1.73m^2 per year per gram of table
-0.06/-0.14 = 45% of the total effect is mediated by copeptin ($p<0.001$). Analysis adjusted for baseline eGFR, age, sex and DNA mutation as

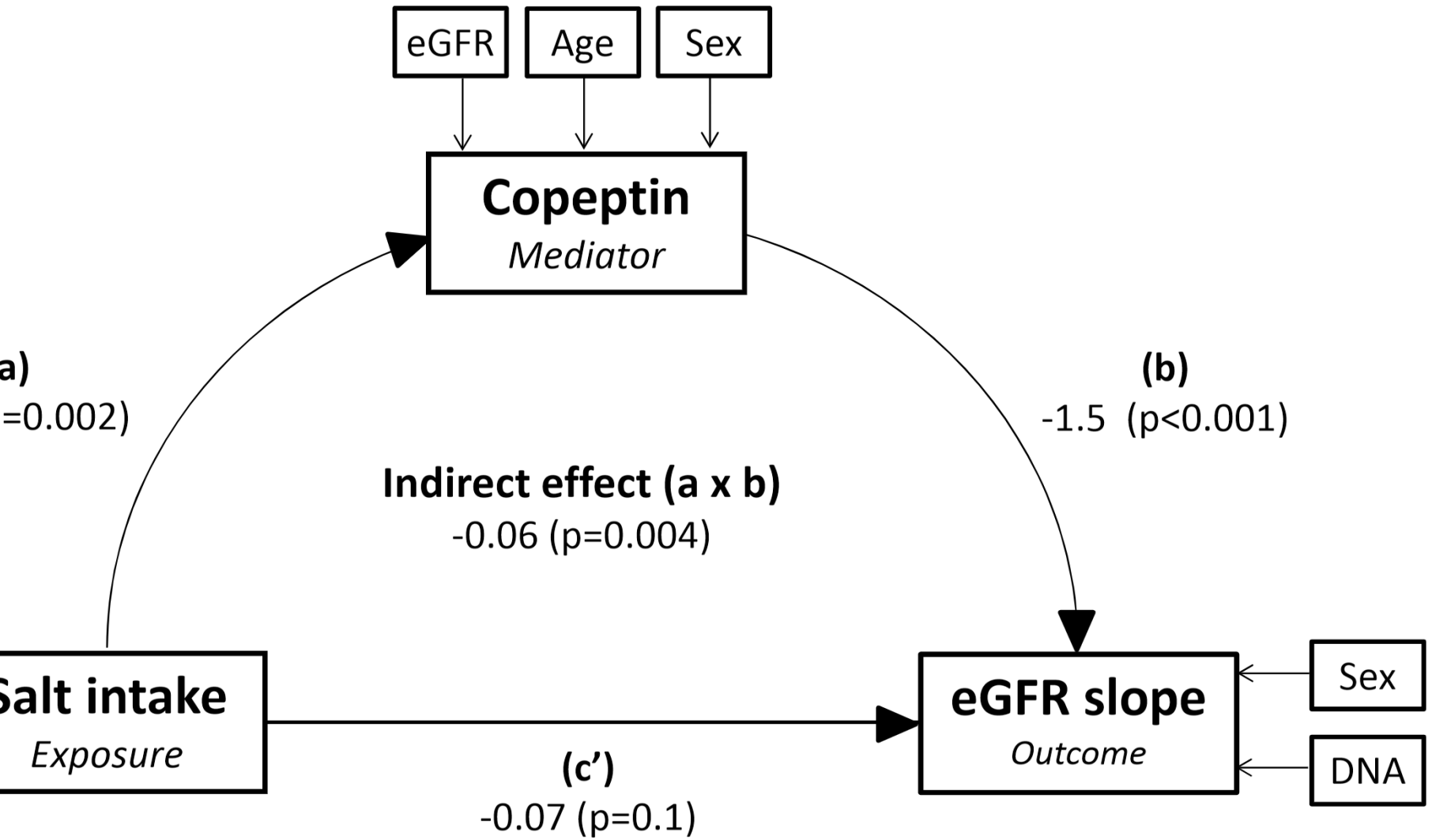

Total participants
n=794
DIPAK-1 participants
n=305
Outpatients
n=489
Excluded (n=17)
<2 years FU n=17
<3 eGFR values n=2
Excluded (n=188)
<2 years FU n=131
<3 eGFR values n=176
DIPAK-1 participants
n=288
Outpatients
n=301
Study sample
n=589


Sodium excretion (mmol/24h)
300
250
200
150
100
50
0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Estimated salt intake (g/24h)
BL
Year 1
Year 2
Year 3
Year 4
Year 5
Year 6
n =
562
548
538
382
294
158
37


Urea excretion (mmol/24h)
700
600
500
400
300
200
130
120
110
100
90
80
70
60
50
Estimated protein intake (g/24h)
BL
Year 1
Year 2
Year 3
Year 4
Year 5
Year 6
n =
562
548
538
382
294
158
37


eGFR slope (mL/min/1.73m²)
-1
-2
-3
-4
-5
-6
60
50
40
30
20
10
0
Frequency
50
100
150
200
250
300
Sodium excretion (mmol/24h)
100
200
300
400
500
600
700
800
Urea excretion (mmol/24h)


Salt intake worse
Salt intake better
n
$P_{interaction}$
Overall
585
Age (year)
>47.5
296
0.06
<47.5
289
Sex
Male
243
0.6
Female
342
eGFR (mL/min/1.73m2)
>60
293
0.07
<60
292
htTKV (mL/m)
>880
276
0.4
<880
277
DNA Mutation
>PKD1
389
0.3
<PKD2/other/no mutation detected
177
Urine volume (mL/24h)
>2200
271
0.1
<2200
276
Plasma sodium (mmol/L)
>140.5
340
0.7
<140.5
241
Plasma copeptin (pmol/L)
>7.5
286
0.3
<7.5
282
RAAS-blocker user
Yes
414
0.02
No
171
Diuretic user
Yes
160
0.4
No
425
-0.50
-0.25
0.00
0.25
Annual eGFR slope per gram of salt
(mL/min/1.73m^2 per year per gram)



Indirect effect (-0.06) / Total effect (-0.14) =

45% Mediation

(

0.04 (p

S